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OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN SOILS FROM *FESTUCA PRATENSIS* HUDS. CROP

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ABSTRACT
Entomopathogenic fungi are the largest group of microorganisms existing in the soil environment. Occurrence and pathogenicity of entomopathogenic fungi in soil is dependent on many factors affecting the soil environment. The aim of this study was to compare the species composition and the intensity of the occurrence of entomopathogenic fungi in soils from monoculture crops of *Festuca pratensis* Huds. The study material consisted of soil samples taken from the experiment conducted in two experimental stations of the Research Centre for Cultivars Study. The insecticides fungi were isolated from soil using a method of the selective substrate. Three species of entomopathogenic fungi *Beauveria bassiana, Isaria fumosorosea* and *Metarhizium anisopliae* were isolated from the study soils using the selective medium.

Keywords: entomopathogenic fungi, soil, meadow fescue.

INTRODUCTION

Entomopathogenic fungi, also known as insecticides, are the largest group of micro-organisms present in the soil environment. Their main advantage is the ability to reduce pests population of agricultural crops, horticultural crops and under cover [Miętkiewski et al., 1991; Quesada-Moraga et al. 2007].

Mostly insect pathogen infection occurs in soil, which is a natural habitat for their existence [Bidochka et al. 1998]. However, existing and pathogenicity of entomopathogenic fungi in soil is dependent on a number of factors affecting the soil environment include temperature, humidity, soil type, structure, and the manner of its use [Tkaczuk, Renella, 2003]. It is essential that the humidity of the soil which determines the possibility of infection, and the temperature which often results in the spreading of some fungi species.

The species composition of fungi as well as their intensity depend on the environment, from which is the soil, and also from the season of year (spring and autumn) and crop species [Gottwald, Tedders 1984; Bałazy 2006].

Insecticides fungi as one of the first pathogens were used in biological pest control in plants. The aim of this study was to compare the species composition and the intensity of the occurrence of entomopathogenic fungi in soils of monoculture crops of *Festuca pratensis L.*

MATERIAL AND METHODS

The study material consisted of soil samples taken from scientific experiments conducted in two experimental stations of the Research Centre for Cultivar Study (COBORU) in Krzyżewo (Podlaskie province) and Uhnin (Lublin province). The samples were taken in two periods: spring and autumn.

The experiment was carried out in two locations and concerned the assessment of yielding of different varieties of tall fescue. Detailed information on the experiment design and soil conditions has shown in Table 1. Samples were taken at random from a 10-th points on a plot. The soil was collected using soil canes to a depth of 15 cm. From the material collected from the area mixed

Table 1. Conditions of experimentation – *Festuca pratensis* Huds.

Lp.	Name of place	Height above sea level (m)	Soil					Forecrop
			Agriculture Value in 100 point scale IUNG	Complex of agricultural suitability	Type	Species	pH 1 n KCl	
	1	2	3	4	5	6	7	8
1	Krzyżewo	130	70	4	A	gl	6,4	Spring barley
2	Uhnin	155	80	1z	M	N	6,6	meadow

Col. 4; 4 – rye very good, 1z – grassland very good and good.
Col. 5: A – podzolic; M – muck.
Col. 6; gl – loam, n – deep peat with thickness of organic matter >30 cm.

sample was performed and stored in plastic bags at 0–4 °C.

Insecticides fungi were isolated from soil using a method of selective substrate developed by Strasser et al. [1996]. The substrate is commonly used for the isolation of entomopathogenic fungi in the soil [Keller et al. 2003, Tkaczuk 2008] .

From each sample originating from a given plot, 2 g was weighed and then 18 ml of distilled water with the addition of 0.05 ml of a solution of Trithon X-100 preparation was added, and vigorously shaken about 35 seconds. Then 0.1 ml of the soil solution was poured in and spread using a glass spatula on a surface of the selective substrate in three Petri dishes, which were replicates. The dishes were placed in incubators at 22 °C and after 8–10 days colonies were counted in each of fungi species. Results were expressed as the number of infectious units (CFU) of entomopathogenic fungi in 1 g of soil.

RESULTS

Using a selective substrate from meadow soils three species of entomopathogenic fungi *Beauveria bassiana, Isaria fumosorosea* and *Metarhizium anisopliae* were isolated. Species composition and units density of the colony-forming of these fungi were varied, depending on the variety and the place from which soil samples were derived. In the soil samples collected in the spring time in Krzyżewo from varieties of tall fescue there were 3 species of fungi. The dominant species was *M. anisopliae* (Figure 1), which formed an average of 1.1×10^3 g^{-1} CFU in 1 gram of soil. *B. bassiana* formed an average of 0.3×10^3 g^{-1}, and *I. fumosorosea* occurred only in soil under Anturka variety. The greatest concentration of CFU of entomopathogenic fungi was observed in the soil from Pasja variety (1.7×10^3 g^{-1}) and Limosa (1.5×10^3 g^{-1}) (Table 2).

In the soil samples derived from the cultivation of tall fescue in Krzyżewo in the second period (autumn), also the dominance of *M. anisopliae* was noted, which created an average of 1.8×10^3 g^{-1} CFU in 1 g of soil (Figure 1). No presence of the fungus *B. bassiana,* and *I. fumosorosea* was noted, it was appeared in the soil under two of the four varieties, forming an average of 0.3×10^3 g^{-1} infectious units per 1 gram of the soil (Table 3, Figure 1).

In the soil samples collected in the spring at Uhnin from tall fescue cultivation a higher density of CFU of the fungus *M. anisopliae* was reported than in autumn. This fungus formed an average of 1.8×10^3 g^{-1} of colony forming units in 1 g of soil, and *B. bassiana* average of 1.3×10^3 g^{-1} (Figure 2). *I. fumosorosea* fungus appeared only in the soil under Anturka vari-

Table 2. The density of infectious units (CFU × 10^3 g^{-1}) of entomopathogenic fungi in soil under cultivation of different varieties of tall fescue (Krzyżewo spring)

Variety	Species of fungi			
	Beauveria bassiana	*Isaria fumosorosea*	*Metarhizium anisopliae*	Total
Limosa	–	–	1.5f	1.5
Pasja	0.7b	–	1.0d	1.7
Anturka	0.2ab	0.2ab	0.7c	1.1
And 1009	0.2ab	–	1.2d	1.4

The value marked with the same letters do not differ significantly.

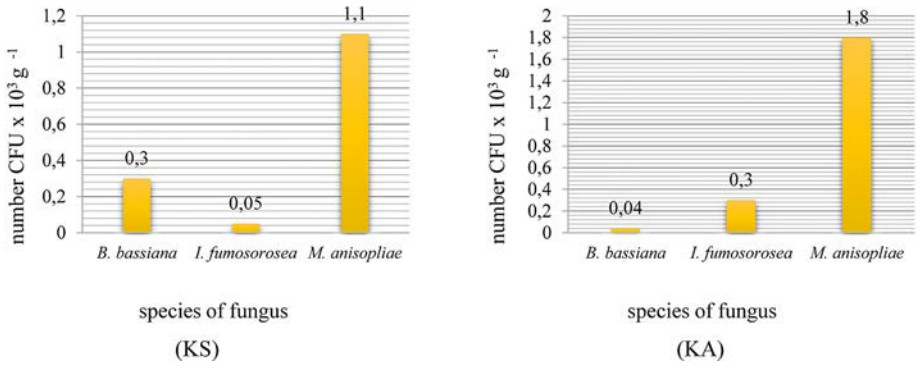

Figure 1. Average concentration of infectious units (CFU × 10^3 g^{-1}) of individual entomopathogenic fungi in soil from cultivation of tall fescue (Krzyżewo – K, spring – S, autumn – A)

ety. Insecticides fungi formed together the most CFU in the soil from plots where varieties Limosa (6.5×10^3 g^{-1}) and Pasja (3.8×10^3 g^{-1}) were cultivated (Table 4).

In the soil samples collected in autumn in Uhnin under cultivation of different varieties of tall fescue the most CFU fungus *B. bassiana* was formed. This species occurred in all soil samples from four cultivated varieties and formed an average of 2.7×10^3 g^{-1} CFU in 1 g of soil (Figure 2).

The fungus *M. anisopliae* formed their colonies only in two from four tested soil samples and *I. fumosorosea* was found only in one sample from the cultivation of Limosa. Insecticides fungi form together the most infectious units in soils under crop varieties Pasja and and 1009 (Table 5).

DISCUSSION

Isolated from meadow soil, the species of insecticides fungi were the most common in the soil environment. This is confirmed by numerous studies carried out by other authors [Keller et al 2003, Bidochka et al 1998, Klingen 2000, Ali-Shtayeh et al 2002, Tkaczuk and Renella 2003, Quesada-Moraga et al 2007, Sun et al 2008].

Using a selective substrate from the soils, on which various species and varieties of grasses were cultivated, three species of entomopathogenic fungi: *Beauveria bassiana, Isaria fumosorosea* and *Metarhizium anisopliae* were isolated. Species composition and units density of the colony-forming of these fungi was varied and depending on the species, variety of grasses, seasons of the

Table 3. The density of infectious units (CFU × 10^3 g^{-1} soil) of entomopathogenic fungi in soil under cultivation of different varieties of tall fescue (Krzyżewo, autumn)

Variety	Species of fungi			
	Beauveria bassiana	*Isaria fumosorosea*	*Metarhizium anisopliae*	Total
And 1009	–	–	3.7h	3.7
Limosa	–	0.4b	0.4b	0.8
Pasja	–	0.2ab	1.4ef	1.6
Anturka	–	–	1.4ef	1.4

The value marked with the same letters do not differ significantly.

Table 4. The density of infectious units (CFU × 10^3 g^{-1}) of entomopathogenic fungi in the soil under cultivation of tall fescue (Uhnin, spring)

Variety	Fungi species			
	Beauveria bassiana	*Isaria fumosorosea*	*Metarhizium anisopliae*	Total
Pasja	3.5h	–	0.3b	3.8
And 1009	1.3ef	–	–	1.3
Limosa	–	–	6.5i	6.5
Anturka	0.3b	0.2ab	0.3b	0.8

The value marked with the same letters do not differ significantly.

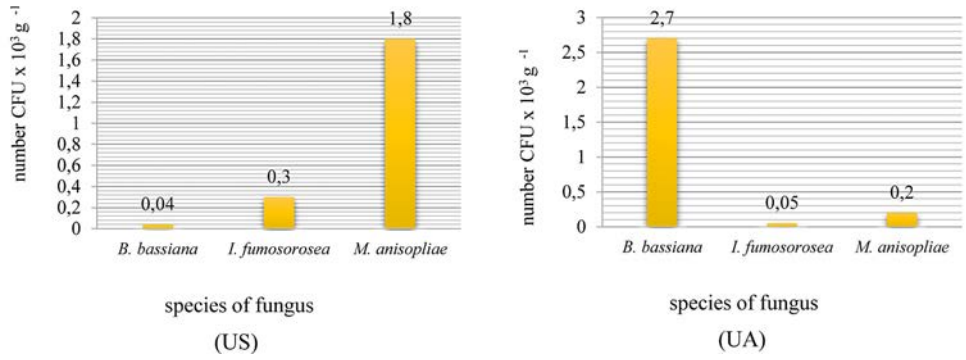

Figure 2. Average concentration of infectious units (CFU × 10^3 g^{-1}) of individual entomopathogenic fungi in soil from cultivation of tall fescue (Uhnin – U, spring – S, autumn – A)

Table 5. The density of infectious units (CFU × 10^3 g^{-1}) of entomopathogenic fungi in the soil under cultivation of tall fescue (Uhnin, autumn)

Variety	Fungi species			
	Beauveria bassiana	*Isaria fumosorosea*	*Metarhizium anisopliae*	Total
Pasja	3.7h	–	–	3.7
And 1009	3.7h	–	–	3.7
Limosa	1.4ef	0.2ab	0.4b	2.0
Anturka	1.9g	–	0.4b	2.3

The value marked with the same letters do not differ significantly.

year and the place from which soil samples were derived. In the soil samples taken on two dates in Krzyżewo, from all grass species and varieties, the fungus *M. anisopliae* was dominated. In Tkaczuk's research [2008], in the national meadow and pasture soils the most common is fungus *M. anisopliae* (present in 96.3% of samples), and then *B. bassiana* and *I. fumosorosea* (50% of the samples). *M. anisopliae* also struck the most *G. mellonella* larvae in these soils. This species seems to be characteristic for meadow and pasture environments and was previously listed as dominant in the soils for meadows and pastures in the UK [Chandler et al 1997], Switzerland [Keller et al 2003] and Australia [Rath et al 1992]. In the soil samples collected in spring and autumn from different species and varieties of grasses in Station of Variety Assessment in Uhnin the most CFU fungus *Beauveria bassiana* was formed. It should be noted that this soil was characterized by a high content of organic matter, in contrast to light and mineral soil from Station Variety Assesment COBORU in Krzyżewo. Research of some authors point to a higher abundance of grassland soils in insecticides fungi than adjacent soils of farmland. Miętkiewski et al. [1992] conducted a study to compare the existing of entomopatogenic fungi in the soil arable and meadow.

In the soil derived from meadow showed significantly greater intensity of *M. anisopliae* than in the soil of cultivated fields. Also, other species with the exception of *P. fumosoroseus*, were more numerous in the meadow soil. The differences were not statistically significant, but the tendency to higher occurrence of entomopatogenic fungi in the meadow soil compared with topsoil was significant. Analyzing the density of CFU in the meadow soils in two seasons – spring and fall, it was found that some species of fungi were present in greater numbers in the spring, while others in fall. Tkaczuk [2008] performing a similar analysis of soils, but using a trap method showed that, depending on the season of year (spring and autumn), there are some changes in the frequency of isolation and in the percentage share of each species of entomopathogenic fungi in the mortality of larvae of *G. mellonella* lined to soils from studded environments in Poland.

Treating all the environments and species of fungi, no significant differences in the frequency of their isolation from the soil in spring and autumn was stated. Fungus *B. bassiana* was significantly more frequently isolated from environments soils in spring (93.2% of samples) than in autumn (73.5%), while in the case of the fungus *M. anisopliae* an opposite tendency was found.

Looking at the separate individual environments only in relation to soils from meadows and pastures was found that insecticides fungi including were significantly more isolated in the spring. The fungus *B. bassiana* existed more numerous in soils of cultivated fields in the spring but the other species were isolated with similar frequency in both seasons. Fungi *B. bassiana* and *I. fumosorosea* were recovered significantly more often in spring than in autumn from soil samples of meadows and pastures

CONCLUSIONS

1. Species composition and density of the colony-forming units varied depending on the variety, season of the year and the place from which originate the tested soil samples.
2. From the study soils using the selective medium three species of entomopathogenic fungi *Beauveria bassiana, Isaria fumosorosea* and *Metarhizium anisopliae* were isolated.
3. Regardless of the soil type the most infectious units were created by fungi *Beauveria bassiana* and *Metarhizium anisopliae.*

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